PATENT
Attorney Docket **051530-5003-05-US**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: **Harald Sontheimer *et al.***)
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Application No. **08/980,395** Examiner: **Unassigned**
)
Filed: **November 28, 1997** Group Art Unit: **1642**
)
For: **Novel Method of Diagnosing &**)
Treating Gliomas)

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DECLARATION UNDER 37 C.F.R. § 1.132

I, Howard Levine do hereby make the following declaration:

1. I have served as President of BioProcess Technology Consultants since 1994. I am a specialist in biopharmaceutical process development, manufacturing and engineering, with twenty years of experience in the biopharmaceutical industry. Before founding BioProcess Technology Consultants, I was Vice President of Manufacturing Operations at Repligen Corporation. Before that time, I worked in process development and manufacturing for Amgen, Genentech and Xoma. I am a member of the Editorial Advisory Boards of BioPharm Magazine and Bio/Pharmaceutical Outsourcing Report. I also serve on the Scientific Advisory Boards of DSM Biologics; AsepCo and the Boston Area Chapter of the International Society of Pharmaceutical Engineering (ISPE). I have served as chairman of the Parental Drug Association's (PDA) Task Force on Chromatography Validation, and have lectured extensively on downstream processing and manufacturing in biotechnology. I received my Ph.D. in chemistry from the University of Chicago in 1978 and completed a post-doctoral fellowship at Harvard University in 1980.

2. I have served as a consultant to Transmolecular, Inc. from December, 1999 to present.

3. That as part of my duties as consultant to Transmolecular, Inc., the licensee of the subject application, I am involved in the development of a pharmaceutical composition containing chlorotoxin for the treatment of numerous neurological diseases in humans.

4. I have examined the journal publication cited in the Office Action dated July 18, 2000 (DeBin *et al.*, (1993) Purification and characterization of chlorotoxin, a chloride channel ligand from the venom of the scorpion, Am. J. Physiol. 264, 361-369 (attached as Exhibit A)).

5. The chlorotoxin composition prepared by DeBin *et al.* and administered to arthropods is not one-hundred percent pure and thus contains significant amounts of impurities. DeBin *et al.* isolated chlorotoxin for their experiments in arthropods by an initial large, preparative purification of chlorotoxin from processed venom, pooling fractions containing chlorotoxin from several separate runs. This initial purification was carried out by loading the processed venom on a C18 reverse-phase HPLC column followed by elution with a linear gradient of acetonitrile in 10 mM trifluoroacetic acid (TFA). The pooled fractions from the initial purification were subjected to a second purification step under identical chromatographic conditions except a smaller fraction size was collected in an attempt to remove further impurities. This material was used for all arthropod toxicity experiments.

As DeBin *et al.* point out, however, impurities still remained in the isolated chlorotoxin preparation (see Figure 1B (inset)). Specifically, two additional peaks were isolated in the large peak from which the fractions were collected. These peripheral peaks were later determined not to have any activity and thus were identified as major contaminants of the chlorotoxin material isolated using the aforementioned purification method (see page 366, column two, second paragraph). A composition with such impurities, while suitable for use in toxicity experiments in laboratory animals, is not suitable for therapeutic use in humans, especially via parenteral administration, because of the presence of these unknown impurities. In addition, the level of

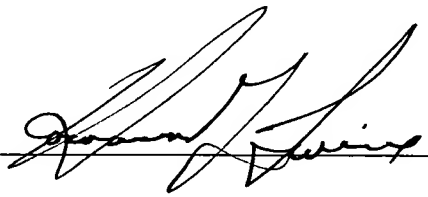
these impurities relative to the level of chlorotoxin is unacceptable for use as a pharmaceutical composition in humans.

6. The chlorotoxin composition prepared by DeBin *et al.* and administered to arthropods also contains high amounts of trifluoroacetic acid (TFA). In their protocol for isolating chlorotoxin, DeBin *et al.* processed the pooled fractions from the second round of chromatography in an identical manner as those in the first round of chromatography. Specifically, fractions containing chlorotoxin pooled and reconstituted in 200-300 ml of 10 mM TFA buffer (see page 364, column 1, lines 2-7). Prior to injection in arthropods, the concentration of the chlorotoxin solution was adjusted by dilution in 10 ml water. Nonetheless, the concentration of TFA in this solution remained at a sufficiently elevated level (mM range) such that it would not be acceptable for administration in humans because TFA is not a pharmaceutically acceptable excipient. Hence, the chlorotoxin composition used by DeBin *et al.* is not acceptable for administration in humans because of the presence of the elevated level of TFA in the composition.

Furthermore, DeBin *et al.* do not indicate that sterile water was used in their arthropod experiments. As is common in acute toxicity experiments such as these, non-sterile water is routinely employed. A pharmaceutical composition containing water that is suitable for parenteral administration in humans requires the use of sterile Water for Injection, produced by distillation of deionized water. The chlorotoxin composition used by DeBin *et al.* is therefore also not acceptable for administration in humans because sterile Water for Injection was not used in the chlorotoxin composition.

7. The chlorotoxin compositions disclosed by DeBin *et al.* are thus not acceptable for human administration because of the presence of impurities and TFA in the composition. The chlorotoxin composition is also not acceptable for human administration because of the presence of non-sterile water in the composition.

I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

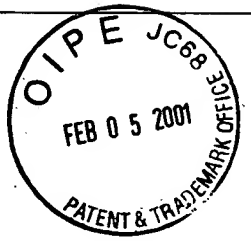
Signed:  Dated: January 22, 2001

Howard L. Levine, Ph.D.
BioProcess Technology Consultants
24 Wright Farm Road
Concord, MA 01742

Curriculum Vitae
Howard L. Levine, Ph.D.

Address: 24 Wright Farm Road
Concord, MA 01742-1528

Phone: 978-371-1733
Fax: 978-371-1738
E-mail: hlevine@bioprocessconsultants.com



SUMMARY

Howard L. Levine, Ph.D. is President of BioProcess Technology Consultants, a consulting firm specializing in biopharmaceutical process development, manufacturing, and engineering. Dr. Levine has over 20 years of experience in the biopharmaceutical industry and was previously Vice President of Manufacturing Operations at Repligen Corporation. He has also worked in process development and manufacturing for Amgen, Genentech, and Xoma. Dr. Levine is a member of the Editorial Advisory Boards of BioPharm magazine and Bio/Pharmaceutical Outsourcing Report. He also serves on the Scientific Advisory Boards of DSM Biologics, a biopharmaceutical contract manufacturing company; AsepCo, a manufacturer of advanced aseptic process equipment; and the Boston Area Chapter of the International Society of Pharmaceutical Engineering (ISPE). He was chairman of the Parenteral Drug Association (PDA) Task Force on Chromatography Validation and has lectured extensively on downstream processing and manufacturing in biotechnology. Dr. Levine holds a Ph.D. in chemistry from the University of Chicago and completed a post-doctoral fellowship at Harvard University.

EDUCATION

1978	Ph.D., Chemistry University of Chicago Chicago, IL Dissertation Title: Flavopapain: Kinetic and Stereochemical Studies of a Semisynthetic Enzyme Thesis Advisor: E. T. Kaiser, Ph.D.
1975	B.S., magna cum laude, Chemistry University of Southern California Los Angeles, CA

PROFESSIONAL EXPERIENCE

1994 – present	President BioProcess Technology Consultants Concord, MA Technical operations consulting, including process development, manufacturing, validation, and facilities design, to the biopharmaceutical industry.
1991 – 1994	Vice President, Product Development (1991 – 1992); Vice President Manufacturing Operations (1992 – 1994) Repligen Corporation Cambridge, MA Responsible for process development, manufacturing and engineering.
1986 – 1991	Director, Pilot Plant Operations Xoma Corporation Berkeley, CA Supervised development, scale-up, and validation of manufacturing processes for monoclonal antibodies.
1984 – 1986	Sr. Process Scientist Amgen, Inc. Thousand Oaks, CA Developed and scaled-up manufacturing processes for recombinant products produced in *E. coli* and mammalian cell culture.
1980 – 1984	Scientist Genentech, Inc. South San Francisco, CA Purified and characterized human proteins produced in *E. coli* and yeast by recombinant DNA.

1978 – 1980	Research Fellow Chemical Laboratories Harvard University Cambridge, MA Conducted structure-function studies of the enzyme Orotidine-5'-phosphate Decarboxylase.

AWARDS AND HONORS

1999	Publisher's Award, Advanstar Publications
1991	Fred Simon Award, Parenteral Drug Association
1978	Marc Perry Galler Prize, University of Chicago
1976 - 1978	National Research Service Award, NIH
1975	American Institute of Chemists Award, University of California
1975	Phi Beta Kappa, University of Southern California

BOARD MEMBERSHIPS

Scientific Advisory Board, ASEPCO Company
Scientific Advisory Board, DSM Biologics
Editorial Advisory Board, Biopharm Magazine
Editorial Advisory Board, Bio/Pharmaceutical Outsourcing Report
Advisory Board, Boston Area Chapter, International Society of Pharmaceutical Engineering

PROFESSIONAL ASSOCIATIONS

American Chemical Society
American Institute of Chemical Engineers
- Treasurer, Northern California Section, 1988 - 1989
- Vice Chairman, Northern California Section, 1989 - 1990
- Chairman, Northern California Section, 1990 - 1991

International Society of Pharmaceutical Engineering
- Member, Boston Area Chapter Advisory Board, 1992 - present

Parenteral Drug Association
- Member, Biotechnology Task Force, 1988 -1992
- Chairman, Biotechnology Task Force, 1990 -1992

SELECTED PUBLICATIONS

H.L. Levine and F.J. Castillo. *Biotechnology: Quality Assurance and Validationl* (K.E. Avis, C.M. Wagner, and V. Wu, Eds.), Interpharm Press, Buffalo Grove, Illinois, p. 51 (1998). Validation of Biopharmaceutical Processes

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R. Wetzel, H.L. Levine, D.A. Estell, S. Shire, J. Finer-Moore, R. M. Stroud and T.A. Bewley. *Interferons* (T. Merigan, R. Friedman, and C.F. Fox, Eds.), UCLA Symposium XXV on Molecular and Cellular Biology, Academic Press, New York, p. 365 (1982). Structure-Function Studies on Human Leukocyte Interferon

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R.A. Hitzeman, F.R. Hagie, H.L. Levine, D.V. Goeddel, G. Ammere, and G.D. Hall. *Nature,* 293, 717 (1981). Expression of a Human Gene for Interferon in Yeast

R. Wetzel, L.J. Perry, D.A. Estell, N. Lin, H.L. Levine, B. Slinker, F. Fields, M.J. Ross and J. Shively. *J Interferon Res,* 1, 318 (1981). Properties of a Human Alpha Interferon Purified from *E. coli* Extracts

H.L. Levine, R.S. Brody and F.H. Westheimer. *Biochemistry,* 19, 4993 (1980). The Inhibition of Orotidine-5'-phosphate Decarboxylase by 1-(5'-phospho-β-D-ribofuranosyl) Barbituric Acid, 6-Azauridine-5'-phosphate, and Uridine-5'-phosphate

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